Progress Report:
Development of Rapid Diagnostic Tests for Measuring Bacterial Indicators in Coastal Waters

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1- EXECUTIVE SUMMARY

Increasingly, the public is concerned about beach closures (i.e., closing beaches to contact recreation) and the safety of swimming at public beaches. A 1995 Santa Monica Bay epidemiological study found a correlation between increased incidences of gastrointestinal illnesses and increased levels of bacterial indicator organisms in storm drain runoff. A direct result of this study was the passage of Assembly Bill (AB) 411 (Chapter 765, Statutes of 1997), increasing the monitoring required for heavily used ocean beaches.

A complicating factor of this required monitoring is the limitation of the current analytical techniques for bacterial indicator organisms. Conventional, culture-based U.S. Environmental Protection Agency (US EPA) approved methods to evaluate recreational waters require an 18 to 24 hour incubation period, while recently published data shows that temporal changes in indicator bacteria levels in beach water occur much more rapidly. This lag time means that a beach with bacterial levels exceeding water quality standards on the day the sample is collected is not posted or closed until at least the following day. This time lag also inhibits tracking of contamination sources, since the signal can dissipate before upstream tracking is initiated. A more rapid analytical method is needed that can be completed on the same day. Therefore, a major element of the State Water Board's Clean Beaches Initiative is the development of a rapid diagnostic method for measuring bacteria indicators in coastal waters.

As part of the State 2001 budget, the State Water Board allocated $1.5 million to fund the development of rapid analytical methods for bacteria indicators in coastal waters. In conjunction with this funding, the California State Legislature enacted AB 639 (Chapter 502, Statutes of 2001) requiring the State Water Board to develop reliable, rapid, and affordable diagnostic tests for measuring bacterial indicators in coastal waters.

In order to meet this mandate in 2002, the State Water Board contracted with the Southern California Coastal Water Research Project (SCCWRP) to conduct this project. The project was organized in phases as follows:

Phase I: SCCWRP organized a workshop on initiating the development of rapid method(s) on May 14-16, 2003 in Monterey. SCCWRP then prepared and distributed a Request for Proposals (RFP) to solicit proposals from researchers throughout the United States. Nine proposals were submitted, and five of these proposals were funded. These methods were evaluated by analyzing blind samples, to demonstrate that the new methods were comparable to the conventional culture-based methods. Two of these methods, Immunomagnetic Separation (IMS) Adenosine Triphosphate (ATP) and Dual Wave Fluorimetry (DWF) performed well enough to proceed to a second round of testing in 2004.

The State Water Board reported the outcome of Phase I of the rapid indicators test development, using the $1.5M at the end of the contract, to the Legislature in 2003 (*Report to the Legislature, July 2003).* SCCWRP continued the testing and evaluation after the contract ended. While funding ceased, State Water Board staff continued to participate in this effort and continued to work with SCCWRP in the development of the rapid methods over the last two years.

Phase II: Several methods demonstrated in 2003, after a year of method development, became sufficiently mature to undergo evaluative testing to assess whether they are suitable replacements for conventional culture-based methods. SCCWRP conducted a study to evaluate the new rapid methods on June 2-4, 2004. The study was designed to demonstrate the new methods and compare them with the conventional culture-based methods, through simultaneous analyses of water samples using both new rapid methods and culture-based methods of enumerating fecal indicator bacteria. The two successful methods from Phase I and two additional methods were evaluated.

Quantitative polymerase chain reaction (Q-PCR) was the most accurate of the methods, but it generally overestimated *Enterococcus* concentration relative to the cultured-based methods. Further, work was necessary to assess the cause for overestimation of *Enterococcus* levels in samples.

Dual Wave Fluorimetry (DWF) had the best precision among the methods and, in some cases, better than the culture-based standard methods. While DWF was very repeatable, it severely overestimated *Enterococcus* count in samples containing urban runoff, either as the matrix or as inoculants.

The Flow Cytometry method registered high results (i.e., 1000 cells/100 milliliter) for almost all samples including most blanks. This is particularly problematic, as it doesn't provide for discrimination between contaminated and non-contaminated sites. Further, development to differentiate between target and non-target cells in this counting process is necessary for this method to succeed.

The Immunomagnetic Separation/Adenosine Triphosphate (IMS/ATP) method had the opposite problem of reporting results near zero for most samples.

While none of the new rapid methods produced results equivalent to those of the conventional culture-based methods, several did perform sufficiently to indicate that they could improve in the near future.

Phase III: SCCWRP recently held another evaluation study on June 21-23, 2005. The study approach was similar to the 2004 Rapid Methods Evaluation Study, which involved demonstrating equivalency with conventional culture-based methods through simultaneous analyses of water samples, using both new and conventional culture-based methods of enumerating fecal indicator bacteria. The samples include both natural samples and laboratory-created samples to ensure that a range of conditions is evaluated.

Seven organizations employing seven methods participated in the 2005 evaluation study. These included the two original methods (IMS/ATP and DWF), which were evaluated in Phase I and Phase II testing, one method (Flow Cytometry) and three variations of another method (Q-PCR), which were evaluated in Phase II, and two new entries (Immunological Dipstick and Transcription Mediated Amplification). All participants analyzed 54 blind samples consisting of triplicates of each of 18 different test samples. Six local laboratories analyzed samples at the same time using conventional methods for comparative purposes

Data evaluation is currently underway and is focusing on the following criteria: Accuracy, Sensitivity, Precision, and Robustness.

The data evaluation will also consider that some of the new methods measure molecular material (e.g., DNA) and will not always produce results equivalent to that of traditional methods, which quantify only live bacteria. To address this concern, the data evaluation will also consider whether the molecular methods are demonstrating correlation with existing methods within sample sets in which the same inoculants are used at three different concentrations. Similarly, the data evaluation will consider whether the new methods produce results that are more equivalent to conventional culture-based methods for samples containing the laboratory strains, since this was selected as inoculants that would maximize the percentage of viable cells.

Proposed Phase IV

After the results of the 2005 Study are evaluated, the appropriate rapid method(s) will need to be refined further, assessed and validated. This should include additional beach water quality testing (including other matrices not already tested such as other marine/estuarine beaches in central and northern California, and fresh water beaches). These new rapid methods must then be certified for use in ambient waters, and the technology will need to be shared and transferred to those who will be using the new methods. This is a critical step in implementing an effective and efficient rapid detection approach for beach monitoring statewide.

This project has been extremely successful to date. It is now not only timely, but it is also essential for the protection of public health and the advancement of beach water quality science to complete this project. Complete development, certification and implementation of the new rapid methods will require concerted State Water Board staff involvement and additional state funding. It is estimated that about $1-2 million will be required for SCCWRP to complete this work. Staff recommends that this funding be made a priority in the Prop 50 Coastal Nonpoint Source Pollution Control Grant Program.

2- PROJECT OBJECTIVE

The objective of this project is the development and evaluation of test methods that measure bacteria level rapidly to make possible a same-day health risk warning. AB 639 (Chapter 502, Statutes of 2001) requires the State Water Board to develop reliable, rapid, and affordable diagnostic tests for measuring bacterial indicators in coastal waters. To address this mandate, the State Water Board contracted with SCCWRP to facilitate the development of rapid tests that measure bacteria levels within hours.

3- PROJECT SCOPE

This project began in 2003 after the execution of the contract with SCCWRP. SCCWRP subsequently developed partnerships with several organizations pursuing a variety of

technological approaches toward same day measurements of indicator bacteria. The State Water Board reported the outcome of Phase I of the project using $1.5M, at the end of the contract period, to the Legislature on July 2003 *(Staff Report To Legislature, July 2003).* SCCWRP continued the testing and evaluation after the contract ended. While funding ceased, State Water Board staff continued to work with SCCWRP in the development of rapid methods over the last two years.
For the purpose of continuity and to provide background information, this report presents a summary of the Phase I (2003 report) and an update on progress of this project. The project was organized in three phases:

Phase I: Summary of the July 2003 Report to Legislature (using the $1.5M State funds)
Phase II: The 2004 Evaluation Study
Phase III: Preliminary update of the 2005 Evaluation Study

The original contract with SCCWRP described the work to be done in phases, phase 1 and phase 2. The project has evolved since it was originally conceived and these original phases 1 and 2 have since been merged into what is now being referred to as Phase I. Additional studies to develop rapid methods continued after the end of the contract and the project grew to include two additional studies that are now referred to as Phase II and Phase III as described in this report. Phase II and Phase III were conducted after completion of the contract.

PHASE I: SUMMARY OF THE 2003 REPORT

SCCWRP prepared and distributed a Request for Proposals (RFP) aimed at researchers throughout the United States who were actively working on rapid microbiological measurement methods for other industries, such as drinking water, food service, counter-terrorism or freshwater ambient monitoring. Nine proposals were submitted. A workshop was conducted on May 14-16, 2003 in Monterey. During this workshop 15 researchers, including the five that eventually received State Water Board funding, made presentations on their individual methods. The attendees included representatives from the Department of Health Services (DHS), the State Water Board, the environmental group Heal the Bay, county health agencies, universities, industry, and New Jersey and Hawaii water quality agencies. The focus of this group was to define the technical, administrative, and financial obstacles to these new technologies, and the best approaches to overcome these obstacles. Based on what was discussed over this workshop, the State Water Board, SCCWRP, and a technical advisory committee worked with the contractors to design the studies necessary to develop a rapid indicator method (s) using the minimum criteria for ranking listed below:

The method would be ready for laboratory testing before April 2003.
The method will detect viable indicator organisms or a molecular substructure of the organism that can be related to the viability of the indicator bacteria.
The method detection limit will allow measurement of bacterial concentrations at or below DHS and the California Ocean Plan bacterial standards.

The analysis can be completed within a normal workday.
The method will be practical and simple to use without extensive training.
The cost of the method will be approximately the same as current analytical costs ($25 - $50 per sample).

Five proposals were selected and funded. A list of the proposed methods and participants is presented in Table 1. These proposed methods are summarized below.

Table 1. Methods Evaluated in 2003 Study

Method	Participant
Immunomagnetic Separation/ATP	University of Michigan
Immunoassay-based Biosensor System	Research International
Dual Wave Fluorimetry	University of Connecticut
Laser-based Optical system	Advanced Analytical Technology
BioAnalyzer with Fluorescence Technique	Sub-Chem

Immunomagnetic Separation (IMS) Adenosine Triphosphate (ATP)

Immunomagnetic Separation/ (IMS) Adenosine Triphosphate (ATP) method was proposed by the University of Michigan. This procedure begins with filtration of the sample. After filtration, the bacteria captured on the filter are resuspended into solution in test tube containers. Antibody-coated beads are added to the suspension. During this time, target bacteria (E.coli) attach themselves to the beads, tagging the E.coli. The test tubes containing the tagged bacteria are inserted into magnetic separators, which separate the tagged bacteria from the liquid. The liquid is poured off, and the tagged bacteria are treated with an agent that ruptures cell walls and releases ATP from each cell. ATP is the major energy source within cells that drives a number of biological processes. Two chemicals are added, which react with the ATP and result in the formation of bioluminescence. The resulting light development is read. The amount of light is proportional to the concentration of bacteria present in the sample. While this method provides good sensitivity, additional work was needed to reduce the time of analyses. The entire procedure should be completed within an hour.

Immunoassay-based Biosensor System

The Immunoassay-based Biosensor System was proposed by Research International. A battery-operated pump is used to draw a water sample through a filter mounted at the pump inlet. The filter is loaded into a cup and the cup is vibrated to loosen the bacteria from the filter. The sample cup is then mounted on a motorized rotation stage, and a waveguide is immersed in the cup, taking a measurement of baseline, and is then removed. The culture media is then transferred to the reagent cup containing an antibody tagged with florescent molecules. The antibody/culture media mixture is incubated for three to six minutes. Then the waveguide is

returned to the cup, and the signal is measured again. The signal level above the baseline measurement is proportional to the number of bacteria in the sample.

At this point, the waveguide is removed and a heater jacket is placed around the sample cup to allow for growth of enterococci. Ideally, this growth period will be less than six hours, allowing sample analyses to be completed within an eight-hour workday.

Based on the results produced in 2003 the Immunoassay-based Biosensor System did not receive funds in Phase II because this method was not as far along as the other three methods.

Dual Wave Fluorimetry

The University of Connecticut is developing an analytical method using fluorescence. The method is based on recent work that several University scientists have patented. Under appropriate conditions, certain enzyme substrates exhibit fluorescence in their unmetabolized state. When the substrate is metabolized, the fluorescent spectrum shifts to a lower frequency. In this method, a substrate capable of fluorescence is added to the sample to be tested. As the target bacteria break down the substrate, the amount of intact substrate decreases as fluorescent emission occurs. By simultaneously monitoring the fluorescent intensity at both emissions bands (the original and the lower frequency), the concentration of bacteria present in the water sample can be determined. Sample analysis should be in the range of two to four hours using this method. Dual wavelength fluorimetry (DWF) is less susceptible to interferences from environmental contaminants because of detection of substrate and products would be affected equally leaving the ratio unchanged regardless of turbidity or the presence of colored substances. DWF had the best precision among the methods and in some cases, better than the laboratories' conventional standard methods. While DWF was very repeatable, it severely overestimated *Enterococcus* count in samples containing urban runoff, either as the matrix or as inoculants.

Laser-based Optical System.

Laser-based Optical System was proposed by Advanced Analytical Technologies (AAT). Water samples are passed through a filter in order to concentrate the organisms present in the sample. The bacteria are then back flushed from the filter into a collection tube, with a goal of recovering greater than 90 percent of the organisms from the filter. A technique called immunomagnetic separation is used to isolate Enterococcus and *E. coli* from other bacteria in the collection tube. Enterococcus and *E. coli* are then combined with fluorescent material. Once the target bacteria are tagged, they can be counted using a specialized cytometer. This instrument focuses a laser beam on the tagged bacteria. Because these bacteria are fluorescently tagged, each individual bacterium emits light, which is collected into a detector tube and processed to give a numeric value. AAT participated in all phases of the 2003 exercise, but its detection limit was never less than 100 cells/100mL. AAT decided to drop development for this application and concentrate on their pharmaceutical product line.

BioAnalyzer with Fluorescence Technique

Sub- Chem Systems has developed a prototype instrument that uses a modification of a technology approved by U.S. EPA for microbiological analyses. Both U.S. EPA and the National Oceanic and Atmospheric Administrative Sea Grant Program have provided funding to Sub-Chem for external evaluations of the prototype instruments. The researchers are adapting a currently used analytical method, which uses fluorescence to quantify indicator bacteria. The submersible BioAnalyzer will take a water sample, incubate it at the correct temperature, and read and transmit the results to a computer. The goal of Sub-Chem Systems is to develop an instrument that can be remotely deployed for near real-time measurement of bacterial indicator organisms, possibly in less than 30 minutes. The instrument could either be attached to a fixed position in the water or allowed to travel with ocean currents. Sub-Chem Systems did not receive funds for method development in Phase II because their methods were not well developed as the other methods. Sub-Chem is still working on their device to make further improvements.

Additional work was conducted on these methods that were presented and evaluated in 2003. These methods became mature to undergo evaluative testing to assess whether they may become suitable replacements for existing conventional methods. SCCWRP scheduled an evaluative testing in June 2004.

PHASE II: 2004 EVALUATION STUDY

Study Design: The study was designed to demonstrate the new methods and compare them with the existing culture-based methods. This was done through simultaneous analyses of water samples using both new and existing methods for enumerating fecal indicator bacteria. The samples include both natural samples and laboratory-created samples, to ensure that a range of conditions is evaluated. Laboratory-created samples offer the ability to control the number of indicator organisms and potentially interfering contaminants present, but cannot completely mimic natural conditions. Environmental water samples were used because they contain complex combinations of interferences that cannot be duplicated in artificial samples, though they offer less control over specific variables that need to be evaluated.

Samples and Methods: Participants analyzed 54 blind samples consisting of triplicates of each of 18 different test samples using the new methods. Five methods including three new methods were evaluated. Table 2 provides a list of these methods and participants. Six local laboratories analyzed these samples at the same time using the conventional culture-based methods for both Enterococci and *E.coli.* A list of the local laboratories is presented in Table 3.

Table 2. Methods Evaluated in the 2004 Evaluation Study

Method	Participant
Immunomagnetic Separation/ATP	University of Michigan
Quantitative PCR	USEPA
Dual Wave Fluorimetry	University of Connecticut
Flow Cytometry	Advanced Analytical Technology

Immunomagnetic Separation (IMS) Adenosine Triphosphate (ATP)

This method was first evaluated in the 2003 Evaluation Study and is described above. Potential end-users who participated in the Phase II evaluation did like the simplicity of the concept behind this method and the fact that, unlike the other methods tested, it was designed for field use. However, it was also determined that this method is labor intensive, and some streamlining of the protocol would be necessary to adopt the method for processing multiple samples in a laboratory.

Quantitative Polymerase Chain Reaction (Q-PCR)

This method detects and enumerates unique genetic targets found in Enterococcus. The bacteria are first captured on a filter. The filter containing the bacteria is then subjected to bead beating, which mechanically lyses the cells and releases their chromosomes (i.e., DNA) into solution. This DNA is then used in the quantitation step, where enterococcal DNA is simultaneously amplified and measured using the Taqman system of fluorescent probes and the advanced optics of the Q-PCR instrument.

Dual Wave Fluorimetry(DWF)

This method was first evaluated in the 2003 Evaluation Study and is described above. It was determined that performing this method is labor intensive and the current configuration of the instrument required considerable set-up times. These shortcomings may easily be overcome by automating certain steps of the test protocol.

Flow Cytometery (FC)

The Flow Cytometry (FC) method was first used in 2003 Evaluation Study and is described above. A concern with this method was the amount of time it took to complete all the necessary sample preparation steps before the sample can be analyzed. However it may be possible to automate many of these steps in order to expedite the process of sample preparation.

Table 3. Local Laboratories Participated in 2004 Evaluation Study

Los Angeles County Sanitation Districts
City of Los Angeles
Orange County Sanitation District
Orange County Public Health Laboratory
City of San Diego
MEC Analytical

Data Evaluation: Q-PCR was the most accurate of the methods but it generally overestimated Enterococcus concentration relative to the conventional culture-based methods. While measurement of noncultive cells is a likely factor in this method's overestimation, other possible

explanations include specificity of the primer set to *Enterococcus* species or that it is a more inclusive measure of *Enterococcus* than the conventional culture based methods. Further work will be necessary to assess the causes for overestimation. However, overestimation is a more preferable problem than underestimation of Enterococcus.

DWF had the best precision among the new methods and in some cases, better than the laboratories' conventional methods. Comparability of DWF with conventional methods was sample dependent. Results were most comparable with conventional methods for samples consisting of a natural seawater matrix with moderate to high level of bacteria. However it severely overestimated Enterococcus counts (i,e,. a high number of false positives) in samples containing urban runoff, either as the matrix or as an inoculum. This was true even when the urban runoff sample had been filtered, suggesting that non-biological process may be responsible for cleavage of the chromogenic substrate in these samples. Further tests will be necessary to evaluate the hypothesis, as urban runoff contamination is an important concern that motivates beach water quality monitoring in southern California.

The FC method registered high values of Enterococcus (i.e. 1000 cells/ 100 mls) for almost all samples relative to the conventional methods and even most of the blanks, leading to a false positive rate of more than 50%. This is particularly problematic, as it doesn't provide for discrimination between contaminated and non-contaminated sites. The overestimation could arise at several places in the measurement process, including attachment of the antibodies to non-target organisms, or incorrect identification of non-cellular materials such as suspended solids. Further work to differentiate between target and non-target cells in this counting process is probably necessary for this method to succeed. Variability among sample replicates was low; therefore this method displayed a high level of precision.

IMS/ATP method had an opposite problem, consistently underestimated the level of *Enterococcus* in the samples, which resulted in a high rate of false negatives. This could have been due to poor antibody recognition of the target, but that is unlikely because this method has produced results comparable to existing methods in previous fresh water testing. A more likely explanation is failure of the magnetic system used to capture bacteria after antibody attachment. Low values in all samples can also result from failure in the ATP quantitation system. Additional research was needed to assess success at each stage in the capture and measurement system.

While none of the new rapid methods produced results equivalent to those of the conventional culture-based methods, several performed sufficiently to cause optimism that they could be sufficiently improved in the near future. Testing also revealed areas of concern that require further method development and evaluation, including how results are affected by constituents of urban runoff in samples or by the presence of high levels of suspended solids.

Participants in this test indicated that their impetus to continue investing in method development hinged upon having a neutral testing forum that provides a mechanism for acceptance of their methods by state/and/or federal regulators. Several participants in the previous evaluation, including the developers of the Q-PCR, DWF and IMS/ATP methods, indicated a willingness to participate in an additional round of testing. In addition, several other groups developing rapid

detection technologies had also approached SCCWRP about inclusion of their methods in future tests.

SCCRWP held an Evaluation Study in June 2005 to further evaluate the available methods.

PHASE III: PRELIMINARY SUMMARY OF 2005 EVALUATION STUDY

Study Design: The study approach was similar to the 2004 Rapid Indicator Evaluation Study. Phase III once again involved demonstrating equivalency with conventional methods through simultaneous analyses of water samples, The samples included both natural samples and laboratory-created samples to ensure that a range of conditions is evaluated.

Sample and Methods: Seven organizations employing five classes of methods have recently participated in the 2005 Evaluation Study. Table 4 presents a list of the methods and particiapants. Table 5 presents a list of the local laboratories that participated by analyzing the samples at the same time using conventional culture-based methods. All participants analyzed 54 blind samples consisting of triplicates of each of 18 different test samples. Table 6 describes the samples analyzed during this workshop. All participants/laboratories analyzed the samples for *Enterococcus* and all local laboratories analyzed the samples for *E. coli*. The participants testing the new rapid methods and the local laboratories recently performed the sample analysis portion of this phase on June 21, 22 and 23, 2005.

Table 4. Methods Evaluated in 2005 Evaluation Study

Method	Participant
Immunomagnetic Separation/ATP	University of California Los Angeles
Quantitative PCR	USEPA NERL
Dual Wave Fluorimetry	University of Connecticut
Multiplex Quantitative PCR	University of North Carolina
Immunological Dipstick	Silver Lake Research
Transcription Mediated Amplification	GenProbe
Quantitative PCR	USEPA Region I

Table 5. Local Laboratories Participated in 2005 Evaluation Study

Los Angeles County Sanitation Districts
City of Los Angeles
Orange County Sanitation District
Orange County Public Health Laboratory
City of San Diego
Weston Solutions

Table 6. Description of Samples Tested in the 2005 Evaluation Study

Inoculants	Matrix	Spiked Concentration (enterococci/100 ml)	Type of Sample
Sewage Effluent	Clean Offshore Seawater	35	Laboratory
Sewage Effluent	Clean Offshore Seawater	104	Laboratory
Sewage Effluent	Clean Offshore Seawater	1000	Laboratory
Urban Runoff	Clean Offshore Seawater	35	Laboratory
Urban Runoff	Clean Offshore Seawater	104	Laboratory
Urban Runoff	Clean Offshore Seawater	1000	Laboratory
Lab Culture	Clean Offshore Seawater	35	Laboratory
Lab Culture	Clean Offshore Seawater	104	Laboratory
Lab Culture	Clean Offshore Seawater	1000	Laboratory
Blank	Sterile PBS	0	Laboratory
Blank	Clean Offshore Seawater	0	Laboratory
Blank	Filtered Offshore Seawater	0	Laboratory
Natural Sample	Doheny Beach at San Juan Creek	Unknown	Wavewash
Natural Sample	Malibu Surfrider	Unknown	Open Beach
Natural Sample	Baby Beach: Dana Point	Unknown	Embayment
Natural Sample	Imperial Beach at Tijuana River	Unknown	Wavewash
Natural Sample	Ballona Wetlands	Unknown	Brackish
Natural Sample	Santa Ana River	Unknown	Urban Runoff

Data Evaluation: SCCWRP is now performing the data evaluation focusing on the following criteria:

- Accuracy: Ability to enumerate indicator organisms in each sample as compared to conventional standard measurement methods.
- Sensitivity: Ability to detect levels of indicator organisms at or below California's regulatory thresholds.
- Precision: Ability to produce comparable values among replicate samples using the same method.
- Robustness: Ability to produce accurate and precise values in different matrices and when interferences are present.

The data evaluation will consider that some of the new methods measure molecular material and will not always produce results equivalent to that of conventional methods, which quantify only viable (live) cells. To address this concern, a determination will be made whether these molecular methods demonstrate correlation with conventional methods within sample sets in which the same inoculants are used at three different concentrations. Similarly, the evaluation will consider whether the new methods demonstrate equivalency with conventional methods for samples containing the laboratory strains of bacteria, since that matrix was designed to maximize the percentage of viable cells.

SCCWRP estimates that the evaluation will be completed and a draft report completed by November 2005.

PROPOSED PHASE IV

After the results of the 2005 Study are evaluated, the appropriate rapid method(s) will need to be refined further, assessed and validated. This should include additional beach water quality testing (including other matrices not already tested such as other marine/estuarine beaches in central and northern California, and fresh water beaches). These new rapid methods must then be certified for use in ambient waters, and the technology will need to be shared and transferred to those who will be using the new methods. This is a critical step in implementing an effective and efficient rapid detection approach for beach monitoring statewide.

Since the end of the original contract, SCCWRP has continued working on this project without dedicated state funding. During this period SCCWRP has estimated spending over $300,000. Significant funding (approximately $2,000,000) has also been contributed by a combination of the U.S. EPA and private companies. These private investments were generated largely as a result of the rigorous scientific evaluation process developed by SCCWRP, the involvement in the process by the State Water Board, and the ultimate process for certification of the successful methods by the State.

This project has been extremely successful to date. It is now not only timely, but it is also essential for the protection of public health and the advancement of beach water quality science to complete this project. Complete development, certification and implementation of the new rapid methods will require concerted State Water Board staff involvement and additional state funding. It is estimated that about $1-2 million will be required for SCCWRP to complete this work. Staff recommends that this funding be made a priority in the Prop 50 Coastal Nonpoint Source Pollution Control Grant Program.

4- REFRENCES

1- *Development of Reliable, Rapid, and Affordable Diagnostic Tests for Measuring Bacterial Indicators in Coastal Waters, State Water Resources Control Board, July 2003*

2- Evaluation of New, Rapid Microbiological Methods for Measuring Recreational Water Quality, John Griffith, Steve Weisberg and Charles McGee, 2004

3- Draft Study Plan To Evaluate New, Rapid Microbiological Measurement Methods For Recreational Water Quality, Southern California Coastal Water Research Project, 2005